

THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

The opinion in support of the decision being entered today (1) was not written for publication in a law journal and (2) is not binding precedent of the Board.

Paper No. 21

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte THOMAS J. CUMMINS, SUSAN M. ATWOOD,
LYNN BERGMAYER, JOHN B. FINDLAY,
JOHN W. H. SUTHERLAND, and JOANNE H. KERSCHNER

Appeal No. 1995-2839
Application 08/062,023¹

ON BRIEF

Before DOWNEY, WILLIAM F. SMITH and ELLIS, ***Administrative Patent Judges.***

ELLIS, Administrative Patent Judge.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 from the final rejection of claims 15 through 27 and 33. Claims 1 through 14, 31 and 32 have been withdrawn from consideration pursuant to 37 CFR § 1.142(b). Claims 28 through 30 have been

¹ Application for patent filed May 14, 1993.

canceled. Claims 15, 17 and 25 are representative of the subject matter on appeal and read as follows:

15. A method for the simultaneous amplification and detection of a first target DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of a first target DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from 7 to 9, and comprising, in the same solution:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of a first target DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequence which are in opposing strands of a second target DNA which is the same as or different from said first target DNA, said third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from 65 to 74E C, all of said primer T_m 's being within about 5E C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymers, a DNA polymerase cofactor and dNTP's, any or all of said additional PCR reagents being supplied in the same or a different composition as defined in i),

to simultaneously amplify said opposing first target DNA strands and said opposing second target DNA strands,

B) simultaneously detecting at least one of said amplified first target DNA strands and at least one of said amplified second target DNA strands as a simultaneous determination of the presence of said first and second target DNA's.

17. The method of claim 15 wherein one or both of said first second primers, and one or both of said third and fourth primers, are labeled with the same or different specific binding moiety.

25. The method of claim 15 wherein said first and second target DNA's are associated with the same or different infectious agent.

The references relied on by the examiner are:

Mullis et al. (Mullis II)	4,683,195	July 28, 1987
Mullis et al. (Mullis III)	4,965,188	Oct. 23, 1990

Mullis et al. (Mullis I), "Specific Enzymatic Amplification of DNA In Vitro: The Polymerase Chain Reaction," **Cold Spring Harbor Symposia on Quantitative Biology**, Vol. LI, pp. 263-73 (1986)

Spaete et al. (Spaete), "Human Cytomegalovirus Strain Towne Glycoprotein B Is Processed by Proteolytic Cleavage," **Virology**, Vol. 167, pp. 207-225 (1988)

Matthews et al. (Matthews), "Analytical Strategies for the Use of DNA Probes," **Analytic Biochemistry**, Vol. 169, pp. 1-25 (1988)

Vandenvelde et al. (Vandenvelde), "Fast Multiplex polymerase chain reaction on boiled clinical samples for rapid viral diagnosis," **Journal of Virological Methods**, Vol. 30, pp. 215-28 (1990)

The claims stand rejected as follows:

I. Claims 15, 16, 20, 21, 27 and 33 stand rejected under 35 U.S.C. §103

as being unpatentable over Vandenvelde, Mullis I and Mullis II.

II. Claims 17 through 19 and 22 through 24 stand rejected under 35 U.S.C. §103 as being unpatentable over Mullis I, Mullis II, Mullis III and Matthews.

III. Claims 25 and 26 stand rejected under 35 U.S.C. §103 as being unpatentable over Mullis I, Mullis II and Spaete.

We **reverse**.

The claimed invention is directed to a method of simultaneous amplification and detection of two target DNA sequences using primers possessing specific properties. As set forth in claim 15, **supra**, the primers must (i) have a T_m (melting temperature) between 65 and 74EC, (ii) have T_m 's within about 5EC of each other, (iii) not differ in length from each other by more than 5 nucleotides, and (iv) hybridize to sequences in opposing strands which are only 90 to 400 nucleotides apart.

Rejection I

The examiner has premised his initial conclusion of obviousness on the combined teachings of Vandenvelde, Mullis I and Mullis II. The examiner states that Vandenvelde discloses (i) rapid simultaneous amplification of multiple target DNA sequences, and (ii) that "the melting behavior of any DNA duplex structure can indeed be predicted from its primary sequence if the relative stability and their temperature-dependent behavior of each DNA nearest-neighbor interaction are known." Answer, p. 6. In addition, the examiner

relies on Mullis I for teaching (i) a method of performing PCR amplification in an aqueous buffer at pH between 7 and 9, and (ii) performing 27 cycles of PCR amplification. **Id.** The examiner relies on Mullis II for disclosing the conditions for optimizing primer length in a PCR amplification reaction. The examiner states that it is her position that “the T_m range would be dependent upon the G:C content of the oligonucleotides [primers] and it would have been obvious to design reaction temperatures within the claimed temperature range.”

Id. The examiner concludes that

It would have been ***prima facie*** obvious to one of ordinary skill in the art at the time the invention was made to detect target nucleic acids by the use of coamplification of target nucleic acid sequences as taught by Vandenvelde et al., combined with primer lengths and T_m ranges as disclosed by Mullis et al. (1987) [Mullis II] and Mullis et al. (1986) [Mullis 1] to achieve methods for the ***simultaneous*** amplification and detection of a target DNA as a whole. (Answer, p. 7)

It cannot be gainsaid that the examiner has the initial burden under § 103 to establish a ***prima facie*** case of obviousness. ***In re Oetiker***, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992); ***In re Piasecki***, 745 F.2d 1468, 1471-72, 223 USPQ 785, 787-88 (Fed. Cir. 1984). To that end, the examiner must show that some objective teaching or suggestion in the applied prior art, or knowledge generally available in the art, would have led those of ordinary skill to combine the teachings of the references to arrive at the claimed invention. ***Pro-Mold and Tool Co. v. Great Lakes Plastics, Inc.***, 75 F.3d 1568, 1573, 37 USPQ2d 1626, 1629 (Fed. Cir. 1996);

In re Fritch, 972 F.2d 1260, 1266, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992).

In the case before us, it does not appear that the examiner has fully appreciated the teachings of the Vandenvelde reference with respect to either the PCR reaction conditions or the desirable properties of the primers. As to the reaction conditions, the examiner's reliance on Mullis I is superfluous. That is, the examiner points to Mullis I for disclosing the number of amplification cycles and pH ranges of the reaction buffer required by the claims, when both these limitations are disclosed by Vandenvelde. As to the T_m ranges of the primers, this, too, is discussed by Vandenvelde, but we find that the examiner presents inconsistent arguments in this regard. That is, on the one hand, the examiner argues that the T_m range is dependent on the G:C content and is a result effective variable and, on the other, that the claimed range is disclosed by Mullis II. The examiner has not cited to any specific section of the reference to support this statement, and none is readily apparent to us.

Neither in his reasons for obviousness, nor in his response to the appellants' arguments, does the examiner rely on what appear to be the most relevant teachings of record. Vandenvelde discloses that

The results of the control experiments involving about [a] hundred PCR-products enable us to say that our FM-PCR protocol has a specificity of 100% even without any final detection assay (data not shown). But this can only be true if the extension primers are correctly chosen. A good amplification primer should form stable duplexes with the target sequence under the annealing conditions, be highly

specific for the intended target sequence (not base-pairing to other regions within the template or to other templates), and not anneal to itself. The critical component in the search for oligonucleotides [primers] which would optimally meet all three of these criteria is the algorithm used for determination of the duplex dissociation temperature (T_m). An algorithm which is commonly used is that of Suggs et al. (1981) where T_m calculation is based on the number of AT and GC pairs.

* * *

Preference should be given to a random base sequence distribution with a [sic, an] average GC-content and a low 5' AT 3' and 5' TA 3' sequences content, the presence of guanosine or cytosine at the 3' (and 5') end, the absence of primer complementarity and secondary structure, and a calculated melting temperature about 60EC by Suggs algorithm and 85EC by Freier-Breslauer algorithm (Freier et al., 1986) [Vandenvelde, p. 225, last para. - p. 226, last para.].

In turning to the specification, we find that the appellants have employed a method of determining the T_m of the primers which is not disclosed by Vandenvelde and which, consequently, results in a calculated melting temperature which differs from those taught by Vandenvelde. Specification, pp. 11-13. We do not find that the examiner has made any attempt, on this record, to make a factual inquiry with respect to these differences and to determine whether the T_m of the claimed primers is the same as, or an obvious variant of, the T_m of the primers taught by Vandenvelde. Thus, we find that the examiner has not established, through the use of factual evidence, or sound scientific reasoning, that the claimed invention would have been obvious to those of ordinary skill in the art at the time the application was filed. A conclusion of obviousness must be based on fact and not on

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unsupported generalities. *In re Freed*, 425 F.2d 785, 787, 165 USPQ 570, 571 (CCPA 1970); *In re Warner* 379 F.2d 1011, 1017, 154 USPQ 173, 178 (CCPA 1967), *cert. denied*, 389 U.S. 1057 (1968). Accordingly, the rejection is reversed.

Rejections II and III

Turning to the remaining rejections in this case, we find that none of the applied prior art teaches the simultaneous amplification of multiple target DNA sequences or the T_m of the primers as required by the independent claim (claim 15); i.e., the claim on which the claims encompassed by this rejection depend. Accordingly, the rejections are summarily reversed.

The decision of the examiner is reversed.

REVERSED

Mary F. Downey)	
Administrative Patent Judge)	
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)	
William F. Smith)	BOARD OF PATENT
Administrative Patent Judge)	APPEALS AND
)	INTERFERENCES
)	
Joan Ellis)	

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